THE ACETYLCHOLINE-LIKE SUBSTANCE IN INSECT NERVOUS TISSUE AND ITS ROLE IN THE TOXICITY OF CHLORINATED HYDROCARBON INSECTICIDES

BY

HENRY ERIC FERNANDO

B.Sc., University of Ceylon, 1947
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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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Introduction

Previous work by Tobias *et al.* (1946) has shown that DDT (2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane) and γBHC (γ isomer of 1,2,3,4,5,6-hexachloro-cyclohexane) cause an elevation of the free ACh (acetylcholine) of the central nervous system of the American roach, *Periplaneta americana* (L.), some hours after the insects had been rendered prostrate by these insecticides. This work was, however, restricted to the central nervous system of the roach, whereas DDT is known primarily for its effects on the peripheral nervous system of insects. The present investigation was therefore embarked upon with a view to study more closely the changes in levels of free ACh in the peripheral nervous system during poisoning of the roach by the chlorinated hydrocarbon insecticides. Bot (1949) found that the hemolymph from roaches prostrate several hours by DDT was toxic to houseflies. A possible relationship between the coincident phenomena of elevated nerve cord ACh and the toxicity of the hemolymph was therefore investigated. The cholinesterase (ChE) activity of tissues of poisoned roaches was studied from the standpoint of its distribution in the body wall, hemolymph, brain, thoracic ganglia and the interganglionic connectives, both because of the effect of DDT on the peripheral nervous system and the marked increase of ACh in the interganglionic connectives of DDT-prostrate roaches noted by Tobias *et al.* (1946). The barbiturates, which are the chief antidotes for poisoning by the chlorinated hydrocarbon insecticides were studied for a possible blocking of the action of ACh, similar to that shown by atropine, since it was felt that the demonstration of such an action by barbiturates might provide corroborative evidence for the involvement of the ACh system during poisoning by the chlorinated hydrocarbon insecticides. The interest in the

nature of the ACh-like substance of insect nerves both from a general standpoint and in its relation to the present investigation prompted a close study of this subject. In view of the finding in this investigation that the substance regarded to date as ACh in insect nerves is not ACh or chemically closely related to it, the biologically active substance in roach nerves is here referred to as the ACh-like substance or as "ACh".

Methods

The chief test insect was the American roach, *Periplaneta americana* (L.). The housefly, *Musca domestica* (L.) and a fleshfly, *Sarcophaga crassipalpis* Macq., were also used in this study. DDT, γBHC and aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene) were the insecticides used and treatment was either topical with acetone solutions or by exposure of the insects to surface residues of the insecticides. The frog rectus abdominis muscle and the heart of the pelecypod mollusc, *Venus mercenaria*, were used for the bioassay of "ACh" from insect nerves. ChE activity was measured in terms of hydrolysis of ACh by using the Hestrin (1949) procedure for the colorimetric estimation of ACh. The blocking of the action of ACh by barbiturates was studied on Venus hearts and the method strictly followed that used for the bioassay of "ACh". The Hestrin (1949) method for the colorimetric estimation of ACh was employed, with some modifications, to study the nature of the ACh-like substance in insect nervous tissue, while the effects of the latter on the central nervous system of the roach, both *in situ* and isolated, were followed with a cathode ray oscilloscope.

Results And Conclusions

The free "ACh" concentration of the central nervous system of the roach increased 140-180 percent after prostration by the three insecticides tested. This elevation of free "ACh" occurred in the brain, ganglia and the connectives. No significant change in the free "ACh" of the central nervous system was observed during the earlier phases of poisoning

but if it did such a change was too small to be observed on account of the wide variation of the free "ACh" levels of the central nervous system of individual roaches. The free "ACh" concentration of the abdominal body wall (probably representing "ACh" of the peripheral nervous system) of male roaches increased 119 percent 30 minutes after knockdown and 2 hours after treatment with DDT, while several hours after prostration by DDT the free "ACh" of the abdominal body wall had increased 293 percent. This marked increase of free ACh in the body wall at an early stage of DDT poisoning may account for the initial effects of DDT as a peripheral nerve poison. In female roaches on the other hand several hours after prostration by DDT the free "ACh" in the abdominal body wall had increased only 30 percent. This obesrvation may find its explanation in the fact that female roaches are far more resistant to the action of DDT than are males. Incubation of normal and toxic hemolymph obtained from normal and DDT, γBHC and aldrin-poisoned roaches respectively with nerve breis gave inconsistant results. The same was true when a brei of the abdominal body wall of normal roaches or hyperactive DDT-treated roaches was used in place of hemolymph in these experiments. However, where a marked rise in free "ACh" was observed only in a few test samples of an experiment of this type, it occurred only where toxic hemolymph or breis of the body wall of DDT treated roaches were used. When the central nervous systems of roaches were bathed *in situ* with normal or toxic samples of hemolymph from untreated roaches or DDT, γBHC and aldrin-treated roaches respectively,elevation in concentrations of free "ACh" in the central nervous system comparable to those obtained in roaches prostrate several hours after topical treatment with the insecticides was observed where toxic hemolymph was used. All this data suggests that the hemolymph from roaches prostrate by treatment with the three insecticides contains a component or components capable of liberating free from bound "ACh" in nervous tissue of the roach *in vitro* or *in situ.*

The ChE activity of the central nervous system, the body wall and the hemolymph remained unchanged during all stages of poisoning of roaches by DDT, γBHC or aldrin. The brain while having the highest "ACh" content was found to have the lowest ChE activity of the various parts of the central nervous system.

The barbiturates which are the chief antidotes for poisoning by the chlorinated hydrocarbon insecticides were found to block the action of ACh on Venus hearts. In this respect they resemble atropine which is the chief antidote for the anticholinesterases. This finding provides further evidence for the involvement of the ACh system during poisoning by the chlorinated hydrocarbon insecticides.

Extracts of the central nervous system of roaches, prepared by McIntosh and Perry's method (1950), which showed biological activity of 28-33 μg./ml. ACh equivalents, were found to contain no ACh on chemical analysis. It is, therefore, concluded that the central nervous system of the roach contains no ACh although the substance present in extracts of roach nervous tissue resembles ACh in most of its biological properties. Extracts of houseflies prepared by McIntosh and Perry's procedure gave yields of the biologically active substance representing only one-tenth that obtained by extraction with eserinized Ringer's solution. This may be due to the destruction of the ACh-like substance of houseflies by trichloroacetic acid, a possibility which would suggest a difference in the nature of the biologically active substance of the housefly and the roach. Such a suggestion is justifiable in view of the difference in the nature of the ChE of the two insects. Injection of extracts of roach nerves, containing about 3×10^{-5} gm./ml. ACh equivalents of the biologically active substance, into the thorax of normal roaches, resulted in almost immediate toxic symptoms resembilng those of DDT and some other insecticides, but with the entire sequence shortened. These effects were more prolonged if treated roaches were maintained at 15° C. than if maintained at 35° C., recovery occurring in about 20 minutes at 35° C. and in 3 to 5 hours at 15° C.

This negative temperature coefficient of action of the "ACh" is accounted for by the temperature coefficient of action of roach nerve ChE. The ACh-like substance, in concentrations of 3 x 10^{-5} gm./ml. ACh equivalents of activity, caused muscular contractions in the isolated leg of a roach and a marked increase of electrical activity of the central nervous system of the roach both when isolated or when *in situ*. Such results are obtained with ACh only when very high concentrations of this ester are used.

Hemolymph, toxic to houseflies, obtained from DDT-poisoned roaches was found to contain 2.5 times more of the ACh-like substance than that found in hemolymph of normal roaches. Injections of dilutions of the ACh-like substance in hemolymph of normal roaches ranging from equality to ten fold that found in hemolymph from DDT-poisoned roaches, into houseflies evoked no abnormal symptoms. Hence, it seems improbable that the toxic action of hemolymph from DDT-poisoned roaches can be attributed to the presence of the increased amounts of the ACh-like substance of roach nerves in it.

DDT and γBHC show a negative temperature coefficient of action while aldrin shows a positive temperature coefficient of action. Temperature coefficients of action of a substance are an expression of the net result of several competitive reactions. In the case of DDT detoxification is greater at higher temperatures, one condition at least favoring a negative temperature coefficient of action. "ACh" is produced in increased quantities during DDT poisoning, and has a negative temperature coefficient of action on roaches. It, therefore, seems that the toxic action of DDT and its negative temperature coefficient of activity as an insecticide can be accounted for on the basis of the liberation of free from bound "ACh", its greater detoxification at higher temperatures, the positive temperature coefficient of action of ChE against "ACh" and the negative temperature coefficient of action of "ACh". The same may hold true for γBHC. Too little is known about the competing mechanisms in aldrin poisoning to warrant any

suggestion of the role of ACh in its action as an insecticide.

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VITA

Henry Eric Fernando was born on April 23, 1923, in Dehiwala, Ceylon. After compelting his early education at St. Peter's College in Colombo, Ceylon, he entered the University of Ceylon, Colombo, Ceylon, in July 1943 to pursue a training in zoology, entomology, and chemistry. In July 1944 he was awarded a Science Scholarship at the University of Ceylon and in January 1945 he was appointed a demonstrator in zoology at the same institution. He served as a demonstrator in zoology until December 1947. He received his B. Sc. degree in July 1947 from the University of Ceylon. In January 1948 he joined the staff of the Division of Entomology, Department of Agriculture, Peradeniya, Ceylon, as an assistant to the Chief Entomologist. After serving one year in that capacity he was awarded a Ceylon Government Scholarship to pursue higher studies in economic entomology in the U.S.A. He joined the Department of Entomology of the University of Illinois as a graduate student in February 1949 and received his M.S. Degree in Entomology in January 1950, from that institution.

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